*wjpmr, 2021,7(13), 48 – 54.*

**SJIF Impact Factor: 5.922**

**WORLD JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH**

www.wjpmr.com

Research Article

ISSN 2455-3301

WJPMR

# ANALYSIS OF AN AEROBIC FUSOBACTERIA USING MASS SPECTROMETRY

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**Article Received on 15/10/2021** **Article Revised on 04/11/2021** **Article Accepted on 25/11/2021**

## ABSTRACT

The aim of this study was to analyze individual polar lipid analogues, within each lipid family present f fusobacteria using fast atom bombardment mass spectrometry (FAB-MS). Polar lipid extracts were prepared, washed and dried. Samples, dispersed in a matrix of *m*-nitrobenzyl alcohol, were analyzed by negative ion FAB-MS using xenon as the reagent gas. Major anion peaks observed in the low mass region of mass/ charge (m/z), 211, 221, 225, 227, 239, 241, 249, 251, 253, 255, 273, 277, 279, 281, 289 and 291 were consistent with the presence of $C_{13:1}$, $C_{14:3}$, $C_{14:1}$, $C_{14:0}$, $C_{15:1}$, $C_{15:0}$, $C_{16:3}$, $C_{16:2}$, $C_{16:1}$, $C_{16:0}$ CM, unknown, $C_{18:3}$,$C_{18:2}$,$C_{18:1}$, CM, unknown and $C_{19:3}$ carboxylate anions. In the high mass region, major anion peaks observed with m/z 644, 646, 648, 660, 662, 672, 673, 674, 686, 688, 689, 690, 698, 700, 701, 703, 714, 716, 717 and 719 were consistent with the presence of phosphatidylethanolamine (PE) (29:2), PE (29:1). PE (29:0), PE (30:1) PE (30:0), PE (3l:2), first isotope of PE (31:2), PE (31:1), PE (32:2) PE (32:1) first isotope peak of PE (32:1), PE (30:0), PE (33:3), PE (33:2) phosphatidylglycerol (PG) (31:3), PG (3l:2), PE (34:2), PE (34:1), PG (32:2) and P. (32:1)We conclude that FAB-MS can provide data on individual analogues of PE and PG from *Fusobacterium* spp. not readily obtained by other means. Furthermore, the phospholipid profile is diagnostic for the genus.

## INTRODUCTION

The genus *Fusobacterium* was proposed by Knorr (1923) for anaerobic Gram-negative spindle-shaped bacilli. The involvement of fusobacteria in a wide spectrum of human infections, e.g tissue necrosis and septicemia, has long been recognized. More recently, their importance in intra-amniotic infections, premature labour and tropical ulcers has been reported (Bennett and Eley 2013). In addition, fusobacteria are also important pathogens in wound infections due to dog and cat bites (Goldstein *et al*. 2017) Because of their asaccharolytic nature and a general lack of reactivity in rou- tine biochemical tests, laboratory identification of fusobacteria proved difficult until the application of novel biochemical and molecular biological techniques (Holdeman *et al*. 2018; Hofstad 2016; Shinjo *et al*. 2019). The latter techniques revealed a number of new species, together with the subspeciation of *Fusobacterium necrophorum* and *F.nuclcatunz*, and provided new means of identification.

Chemotaxonomy techniques (Goodfellow and Minnikin 2015) have also been applied to *Fusobacterium* spp. Gas chrornatography (GC) has been used to analyze metabolic products and constituents such as hydroxy acids, fatty acids and lipopolysaccharides (Dhalen and Ericsson 2016; Carlier 2017; Jantzen and Hofstad 2017; Hofstad and Iantzen 2018). A development has been the use of mass spectrometry (MS) which can provide absolute identification of analytes separated by GC (Drucker and Jenkins 2019). Usually mass spectrometry ionizes analytes either by electron impact MS or chemical ionization MS. An alternative method of ionization is fast atom bombardment (FAB) using inert gas atoms (Barber *et a1*. 2011). The characteristics of FAB are the ability to ionize non-volatile molecules selectively, to ionize surface-active (polar) compounds from the surface of a matrix fluid and to separate mixtures of compounds. Fast atom bombardment MS is suitable for analysis of bacterial polar lipids (Heller *et al.* 2017, 2018) and provides novel date of taxonomic value for a wide for a wide range of mictobial taxa (Drucker *et al* 2014; Drucker *et.al* 2015, 2016). The **Aim of This Study** was to determine the distribution of phospholipid analogues within certain *Fusobacterium* species using mass spectrometry.

## MATERIALS AND METHODS

**Isolates studied.** All isolates of *Fusobacterium* studied (Table 1) were from the collection (Department of Microbiology).

### Sample preparation

Isolates tested were checked for purity and identities were confirmed using microscopic and colonial morphology, gaseous requirements and biochemical reactions (Rapid ID 32A; Bio Merieux SA, Marcy-

I'Etoile, France). Strains were grown as a lawn of growth on plates of 5% (v/v) horse blood-Fastidious Anaerobe Agar (Lab—M, Bury, UK) in a Compact M anaerobe work station (Don Whitley Scientific, Shipley, UK) in an atmosphere of 80% NZ/ 10% COZ/ 10% H2 (v/v) at 37 °C for 48 h. Growth was harvested with a saline-damped cotton wool swab taking great care not to remove any agar. Under these conditions, no peaks were obtained attributable to contamination from swabs or culture medium. Cells were resuspended in 10 ml saline and then pelleted by centrifugation at 3000 g for 20 min. The pellet was washed using distilled water and freeze-dried using a Modulyo lyophilizer (Edwards, Crawley, UK) so that standard dry weights of cells (10 mg) might subsequently be extracted, using chloroform :methanol (112, v/ v), dried and partitioned between 5 ml distilled water and 5 ml Analar chloroform with intermittent shaking over a 4-h period at ambient temperature.

**Table 1: Shows the tested isolates.**

| Species | Strain | Study |
|---|---|---|
| *Fusobacterium nucleatum* | Q6829 | A |
| *Fusobacterium nucleatum* | M2175 | B |
| *Fusobacterium nucleatum* | 57 | C |
| *Fusobacterium necrophorum* | Bacon | D |
| *Fusobacterium ulcerans* | 1000 | E |
| *Fusobacterium ulcerans* | 1000 $_7$ | F |
| *Fusobacterium ulcerans* | 1000G | G |
| *Fusobacterium varium* | VPI 4234 | H |
| *Fusobacterium russii* | VPI 0307 | I |

The chloroform phase was then dried in vacuum over anhydrous calcium chloride to assist removal of water. Samples were briefly stored at -20°C until required for analysis. Gloves were worn and glassware solvent-washed so that environmental lipid would not contaminate samples. All strains were subsequently re-grown and re-processed to confirm reproducibility.

**Phospholipid Analysis**

Dried samples were reconstituted in 10 µ l methanol and then admixed with 10 µ l meta-nitrobenzyl alcohol which acted as the matrix fluid for mass spectrometry. After allowing the methanol to evaporate on a copper probe, samples were placed in the chamber of concept IS mass spectrometer. Polar lipids, which are surface-active and migrate to the surface of the matrix, were preferentially ionized by FAB using xenon atoms. Anionic spectra were recorded and l0 scans were averaged. Data were obtained for ion intensities (relative to the most intense ion) over a range of mass-to-charge (m/ z) values in the range 200-1000. Spectra were obtained additionally in digital form.

**Data Analysis**

The carboxylate anions (mz 200-300) were first putatively identified. These assisted tentative assignment of higher mass ions (550-1000) as being consistent with the expected presence of particular phospholipid molecular species. Anions putatively assigned as carboxylate anions were designated by the shorthand 'x:y', where x = the number of atoms of carbon in a fatty acid molecule and y = the number of unsaturations. The latter refers to either double bonds or cyclopropane rings. For example, 18:1 is octadecanoate which is the anion of a monocarboxylic acid having 18 carbon atoms and one double bond in its carbon chain. For peaks assigned as phospholipids, the following designation was used per family of phospholipid: PE, phosphatidylethanolamine and PG, phosphatidylglycerol. Both types of phospholipid consist of a number of analogues (molecular species) differing in molecular Weight because they do not all have the same fatty acyl substituents. For example, PE (30:1) is a phosphatidylethanolamine having two fatty acyl substituents on glycerol that have a total of 30 carbon atoms and one site of unsaturation. Quantitative analysis followed. In order to facilitate this, the intensities of major peaks were normalized so that (peak intensities) = 100, per strain, for carboxylate or phospholipid anions. Further manipulation of data was possible after entering normalized values into dBASE IV (Borland) files. It was then possible to prepare tables of normalized values, check calculations and export files to SPSS/PC + (DOS) (SPSS Inc.) for further analysis, e.g. calculation of correlation coefficients (Drucker 2015).

## RESULTS

The species of *Fusobacterium* included in this study were all anaerobic Gram-negative rods. Their identities were confirmed using Rapid ID 32 A. The results obtained confirmed the identity of the isolates. Each FAB-MS spectrum in negative ion mode was the mean of l0 scans. The regions of each spectrum of particular interest were the lower mass region (m/z range 200-300) containing peaks attributable to carboxylate anions and the higher mass region (m/z 600-800) containing peaks attributable to anions of expected phospholipid analogues. It was possible to calculate the molecular weight of individual phospholipid analogues from m/z anions (molecular weight = m/z + l). A typical spectrum is shown in Fig.1 for F. *ulcerans.* All isolates were analysed on two separate occasions and mean data are presented in Tables 2 and 3 for carboxylate anions and in Tables 4 and 5 for phospholipid analogue anions. In Tables 2 and 3 major anions range from m/z 211 to 291. The anions were identified putatively on the basis of having a value consistent with the expected presence of particular carboxylate anions. For example, the most intense anions were with m/ z 227, 253,255 and 277 which may be putatively assigned the following identities: $C_{14:0}$ (tetradecanoate), $C_{16:1}$ (hexadecenoate), $C_{16:0}$ (hexadccanoate) and ($C_{18:3}$ (oetadectrienoate). (quantitative differences between isolates were measured using the Pearson Coefficient of linear Correlation (r). A value unity would indicate that two strains were identical. Decreasingly similar strain pairs have smaller values for the coefficient r. 1n the case of *f.ulcerans* (1000), the most closely similar strain was *f.ulcerans*

(1000), r = 094. Generally, however, isolates appeared to have carhoxylate anion profiles similar to those of other species.

Details of phospholipid anion profiles obtained are shown in tables 4 and 5. The major phospholipid (PL) analogues of strains examined were m/z 688, PE (32:1) together with its first isotope peak with m/z 689 and m/z 672, PE (31:2) and its first isotope peak at m/z 673. The other peaks were putatively assigned the following identities :719, PG (32:1); 717, PG (32:2); 716, PE (34:1); 714, PE (34:2); 703, PG (31:2); 701, PG (31:3); 700, PE (33:2); 698, PE (30:0); 690, PE (30:0); 686, PE (32:2); 674, PE (31:1); 662, PE (30:0); 660, PE (30:1); 648, PE (29:0); 646, PE (2911) and 644, PE (2912). When profile for different strains were compared using the Pearson Coefficient of Linear Correlation, it was found that the most similar strain to F. ulcerans 1000y (r = 0:77) and F *ulcerans* 1000G (r = 0.85) was the other F. *ulcerans* strain. Paradoxically, F. *ulcerans* 1000 was marginally more similar to *F. varium* (r = 0.86) on the basis of phospholipids anion profile. *Fusobacterium necrophorum* BACON was extremely dissimilar to isolates of *F.ulcerans* 1000 (r = - 0.16), 1000γ (r = - 0.16), 1000G (r = - 0.04) and to *F. varium* VP14234 (r = - 0.37).

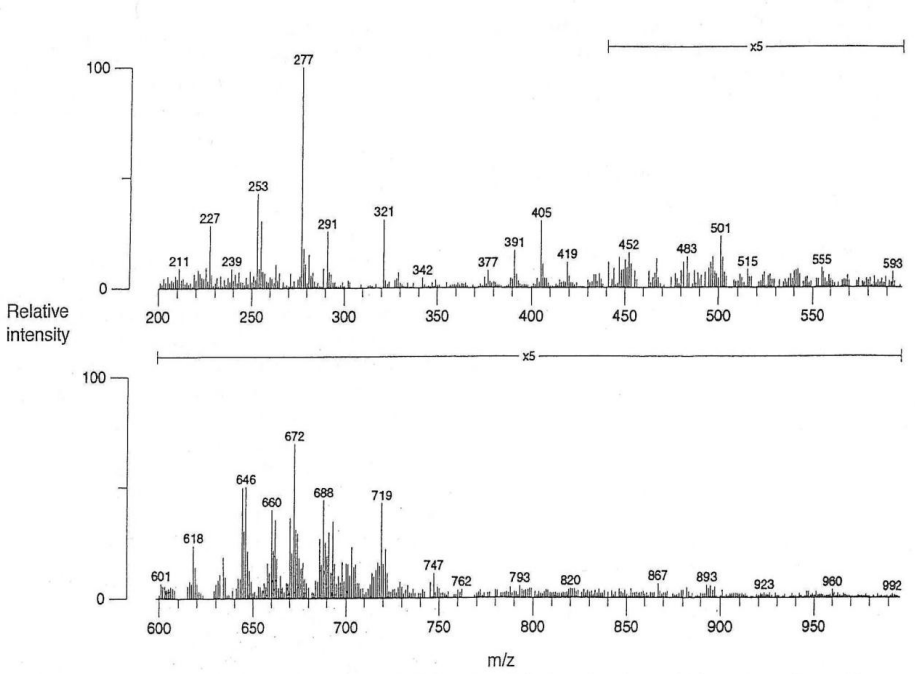


**Fig. 1** Fast atom bombardment mass spectrum showing polar lipid anions of *Fusobacterium ulcerans*. Major peaks are discussed in the text

**Table 2** Negative ion fast atom bombardment mass spectrometry of major carboxylate anions of *Fusobacterium* spp.

| Carboxylate anions | | *Fusobacterium* isolate | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| m\z | ID | A | B | C | D | E | F | G | H | I |
| 211 | 13:1 | 4·1 | 5·6 | 1·7 | 1·3 | 2·8 | 1·8 | 3·0 | 2·1 | 2·8 |
| 211 | 14:3 | 2·5 | 3·4 | 2·0 | 3·4 | 2·4 | 3·1 | 3·2 | 1·4 | 2·7 |
| 225 | 14:1 | 2·9 | 2·7 | 3·6 | 3·4 | 2·6 | 3·5 | 4·1 | 3·6 | 3·3 |
| 227 | 14:0 | 14·9 | 11·6 | 18·4 | 5·8 | 7·2 | 11·0 | 16·9 | 16·3 | 9·5 |
| 239 | 15:1 | 1·8 | 3·1 | 2·0 | 2·8 | 2·3 | 1·1 | 1·8 | 2·5 | 2·1 |
| 241 | 15:0 | 3·8 | 3·9 | 2·0 | 1·5 | 2·3 | 1·3 | 1·6 | 2·0 | 1·5 |
| 249 | 16:3 | 2·3 | 2·2 | 1·1 | 4·3 | 3·9 | 3·3 | 1·4 | 1·0 | 2·1 |
| 251 | 16:2 | 2·2 | 3·8 | 3·1 | 1·6 | 1·2 | 0·8 | 3·2 | 3·1 | 3·6 |
| 253 | 16:1 | 15·6 | 12·5 | 33·2 | 8·0 | 10·2 | 14·5 | 27·1 | 30·4 | 30·2 |
| 255 | 16:0 | 20·3 | 12·1 | 23·6 | 14·2 | 8·3 | 11·6 | 16·6 | 21·0 | 16·1 |
| 273 | U | 1·8 | 2·5 | 0·9 | 1·4 | 2·7 | 1·6 | 2·0 | 1·7 | 2·2 |
| 277 | 18:3 | 9·0 | 20·9 | 1·7 | 30·2 | 29·8 | 26·1 | 8·6 | 5·6 | 8·3 |
| 279 | 18:2 | 3·6 | 2·7 | 2·0 | 5·0 | 3·0 | 2·8 | 2·6 | 2·2 | 3·4 |
| 281 | 18:1 | 9·9 | 4·7 | 3·4 | 6·4 | 3·7 | 4·0 | 3·4 | 3·9 | 8·7 |
| 289 | U | 2·7 | 4·3 | 0·8 | 3·0 | 7·5 | 6·3 | 2·2 | 1·5 | 1·8 |
| 291 | 19:3 | 3·0 | 5·1 | 0·9 | 7·9 | 10·6 | 7·7 | 2·7 | 2·1 | 2·2 |

m\z, Mass to charge; ID, putative peak assignment, e.g. 14:0 is the anion of a 14-carbon fatty acid having no unsaturations in its carbon chain (see text for details); U, unknown peak. For strain identities see Table 1 and text.

**Table 3** Negative ion fast atom bombardment mass spectrometry spectra of major carboxylate anions of strains tested

| *Fusobacterium* strain | Major anions |
|---|---|
| A | 255 > 253 > 227 > 281 > 277 > 211 > 241 > 279 > 291 > 225 |
| B | 277 > 253 > 255 > 227 > 211 > 291 > 281 > 289 > 241 > 221 |
| C | 253 > 255 > 227 > 225 > 281 > 251 > 241 > 211 > 249 > 273 |
| D | 277 > 255 > 253 > 291 > 281 > 227 > 279 > 249 > 221 = 225 |
| E | 277 > 291 > 253 > 255 > 289 > 227 > 249 > 281 > 279 > 211 |
| F | 277 > 253 > 255 > 227 > 291 > 289 > 281 > 225 > 249 > 221 |
| G | 253 > 227 > 255 > 277 > 225 > 281 > 221 = 251 > 211 > 291 |
| H | 253 > 255 > 227 > 277 > 281 > 225 > 251 > 239 > 279 > 211 |
| I | 253 > 255 > 227 > 281 > 277 > 251 > 279 > 225 > 241 > 211 |

For strain identities, see Table 1: for peak identities, see Table 2.

**Table 4: Negative ion fast atom bombardment mass spectrometry of some phpspholipid anions of *Fusobacterium* spp. phospholipid anions *Fusobacterium* species.**

| m\z | ID | A | B | C | D | E | F | G | H | I |
|---|---|---|---|---|---|---|---|---|---|---|
| 644 | PE(29:2) | 4.4 | 5.2 | 3.8 | 0.6 | 8.2 | 9.7 | 9.1 | 12.5 | 3.5 |
| 646 | PE(29:1) | 4.6 | 6.9 | 4.8 | 4.0 | 7.9 | 9.9 | 6.4 | 9.6 | 2.3 |
| 648 | PE(29:0) | 3.6 | 4.6 | 2.2 | 0.0 | 2.4 | 2.5 | 1.7 | 2.3 | 1.9 |
| 660 | PE(30:1) | 4.9 | 4.5 | 7.6 | 3.4 | 7.3 | 9.0 | 10.4 | 4.6 | 8.0 |
| 662 | PE(30:0) | 3.8 | 4.6 | 7.1 | 5.5 | 6.3 | 7.4 | 6.3 | 3.9 | 6.0 |
| 672 | PE(31:2) | 5.0 | 11.2 | 3.1 | 1.5 | 11.1 | 6.5 | 8.5 | 15.0 | 2.0 |
| 673 | PE(31:2)* | 4.0 | 6.1 | 2.6 | 4.0 | 5.5 | 9.4 | 4.6 | 7.8 | 2.0 |
| 674 | PE(31:1) | 3.8 | 4.4 | 2.5 | 7.1 | 5.5 | 5.8 | 4.6 | 6.2 | 2.7 |
| 686 | PE(32:2) | 3.8 | 4.4 | 6.4 | 5.4 | 4.7 | 4.6 | 7.9 | 4.4 | 12.3 |
| 688 | PE(32:1) | 10.0 | 9.6 | 25.9 | 11.9 | 8.1 | 9.2 | 8.9 | 5.7 | 12.7 |
| 689 | PE(32:1)* | 6.9 | 5.4 | 11.6 | 5.4 | 4.7 | 3.8 | 5.5 | 3.4 | 6.3 |
| 690 | PE(32:0) | 5.0 | 4.0 | 6.5 | 4.8 | 4.0 | 3.5 | 2.4 | 3.2 | 4.3 |
| 698 | PE(33:3) | 2.6 | 3.0 | 1.5 | 1.2 | 2.9 | 2.2 | 2.3 | 3.2 | 2.4 |
| 700 | PE(33:2) | 2.4 | 3.8 | 2.2 | 4.5 | 2.6 | 2.1 | 2.4 | 2.7 | 2.9 |
| 701 | PG(31:3) | 3.4 | 3.1 | 2.3 | 5.7 | 2.1 | 2.8 | 2.6 | 3.1 | 2.4 |
| 703 | PG(31:2) | 5.4 | 3.2 | 3.1 | 5.4 | 3.2 | 2.3 | 2.5 | 3.3 | 4.0 |
| 714 | PE(34:2) | 3.5 | 3.6 | 2.6 | 8.2 | 2.2 | 3.4 | 2.1 | 1.9 | 6.7 |
| 716 | PE(34:1) | 8.4 | 3.0 | 2.1 | 8.5 | 2.7 | 2.1 | 2.3 | 1.7 | 6.0 |
| 717 | PG(32:2) | 5.9 | 4.5 | 1.1 | 8.2 | 3.0 | 2.4 | 4.2 | 2.2 | 5.3 |
| 719 | PE(32:1) | 8.9 | 5.4 | 1.9 | 5.1 | 6.2 | 2.1 | 5.7 | 4.7 | 6.8 |

First isotope peak.m/z, Mass to charge; ID, pmk identity; PG(x:y), phosphatidylglycerol analogue with 'x' total carbons in fatty acid substituents and 'y' total unsaturations; PE, phosphatidylethanolamine. For strain identities, see Table 1; for peak identities, see Table 4.

**Table 5 : Major anions in negative ion fast atom bombardment mass spectrometry spectra of phospholipids of strains Tested.**

| | *Fusobacterium* species | Major anions |
|---|---|---|
| A | 688 > 719 > 716 > 689 > 717 > 703 > 690 > 660 > 646 > 644 |
| B | 672 > 688 > 646 > 673 > 689 > 719 > 644 > 648 > 717 > 674 = 686 |
| C | 688 > 689 > 660 > 662 > 690 > 686 > 646 > 644 > 672 > 673 |
| D | 688 > 716 > 714 = 717 > 674 > 701 > 662 > 686 > 719 > 690 > 700 |
| E | 672 > 644 > 688 > 646 > 660 > 662 > 719 > 673 = 674 > 686 > 690 |
| F | 646 > 644 > 673 > 688 > 660 > 662 > 672 > 674 > 686 > 689 |
| G | 660 > 664 > 688 > 672 > 686 > 646 > 662 > 719 > 689 > 673 = 674 |
| H | 672 > 644 > 646 > 673 > 674 > 688 > 719 > 660 > 686 > 662 |
| I | 688 > 686 > 660 > 719 > 714 > 689 > 662 > 717 > 690 > 703 |

## DISCUSSION

When FAB-MS was first used for analysis of polar lipids of bacteria it was belived that there was little fragmentation of molecular anions. It remains true that molecular anion are well preserved which is why complex mixtures can be analysed without prior separation of molecular species peaks (Heller *et al.* 2017),. However, spectra are complicated by the fact that: i) some destruction of molecules does occur and that ii) as molecular weight increases so does the proportion of first isotope anions. The latter are due to

the presence of $^{13}C$ which is a naturally occurring isotope of carbon with an abundance of 1.11%. this is apparent in Fig 1where m/z 225 is followed by a peak with m/z 256 and m/z 719 is followed by a peak with m/z 720. The peak witth m/z 719 has a proportionately higher first isotope peak (at m/z 720) because there is a higher statistical probability that a molecule of a higher rnass phospholipid will contain a $^{13}C$ atorn than is the case with a lower mass carboxylate anion peak (eg. m/z 256). Very small peaks probably represent noise and/ or fragmentation products and are less reproducible than peaks for more intense ions. Prior preparative eparation of phospholipicls by high-performance liquid chromatography would be required (Batley *et al*. 2012) before individual analogues could be separated and prepared for GC analysis. Such work is excessively time-excessively and, although FAB-MS cannot provide such firm peak identification, the technique is m uch faster and simpler for everyday use.

Previously, taxonomic studies have examined the major fatty acid composition of whole cells of *Fusobacterium* spp. (Hofstasd and Jantzen et al. Carlier 2017). It was found that *Fusobacterium* spp. could be differentiated on the basis of qualitative and quantitative analysis of carboxylic acids. The major types of carboxylic acid detected their anions in this study were $C_{12:0}$ (tetradecanoate) $C_{16:1}$ (hexadecenoate) and $C_{18:3}$ (octadectrienoate) which were similar to the acids described by Hofstad and jantzen (2018) and jantzen and I- Hofstad (2017) *Leptotrichia buccalis* and *Fusobacterium* spp. The former study found $C_{16:0}$, (n-hexadecanoate), $C_{18:1}$ (octadecenoate) and 3- OH-$C_{14:0}$ (S-hydroxy tetradecanoate) to be the major carboxylic acids detected by gas-liquid chromatography (GLC) for *L.buccalis*, while the latter study found that *Fusobacterium* species are characterized by the presence of 3-OH- $C_{14:0}$ (3-hydroxytetradecanoate), $C_{14:0}$ (n-tetradecanoate), $C_{16:1}$ (hexadecenoate), $C_{16:0}$ (n-hexadecanoate) and $C_{18:0}$ (octadecenoate). Wardle *et.al*. (2016), using identical FAB-MS methods to the present study, found that the $C_{15}$:$_0$ (pentadecanoate) anion is the most intense carboxylate peak in negative ion spectra of bactericides and that other anions were attributable to saturated, mono unsaturated, di-unsaturated, tri-unsaturated and hydroxy-carboxylate ions. Previously FAB-MS has shown the major carboxylate anion in single strains of *F.necrophorum* and *F. ulaerans* to be m/z 277 compared with m/z 2Z7 (tetra-decanoate) in *Treponenza denticola* or m/z 24-1 (*pentadecanoate*) in *Capnocytaphaga ochracea*, *Porphyromonas gingivalir*, *Pre-votella.bivia* and *Prevotella denticola* (Drucker 2017). In other words, profiles vary considerably from one genus to another. With respect to phospholipid analogue profiles, the most prominent anions were consistent with the presence of PE (31: 2) or PE (32. 1) depending on the *Fusobacterium* spp. In other Gram-negative anaerobes examined, the most intense peaks could be attributed to rather different analogues (Drucker 2017). For example, the most intense peaks were m/z 662, attributable to PE (30:0), in *Bacteroides eggerthii* and in *C.ochracea*. Such differences reflect differences in the composition of major cellular fatty acids between different taxa. Interestingly, the finding of PE analogues as the major type of phospholipid in these Gram-negative bacteria is in agreement with the findings of Wardle *et al*. (2016) who applied the technique to Bacteroides spp. and found PE (30:0), PE (OH-30:0) ، and PE (OH-31:0) to be the major analogues. Thus PE is the major phospholipid type detected in lipid extracts of Gram-negative bacteria examined by FAB-MS (Drucker 2017). This is in contrast to Gram-positive bacteria, such as *Lactobacillus spp*. (Drucker *et.al* 2015), which have PG analogues as their most prominent anions. In other Gram-positive genera, PG is again the major type of PL, e.g. staphylococci (Drucker and Abdullah 2015) and Clostridium difficile (Drucker *et al* 2016) In addition, polar lipids of *Fusobacterium* spp. are more similar to those of enterobacteria (Aluyi *et al*. 1992) than to Gram-positive taxa so far studied, for example, the analogue PE (32:1) is a major peak both in fusobaeteria and enterobaeteria. There was a considerable difference, as might be expected, between the PL alnalogue profiles described here and those reported by Abdi and Drucker 2016 for the oral yeast, *Candida spp.* n the latter study, PE (43: 5), PG (34:3), PG (32:3), PE (36:1) and PE (43:5) were found. In yeasts it would be expected that more highly unsaturated, longer chain, acids would be present than in fusobacteria. For the isolates of *Fusobacterium* spp. studied here, the PE analogues constituted a considerably larger proportion of polar lipid than did PG analogues. Comparison of this finding with other studies is complicated by the lack of standardization between laboratories and the use of different techniques. However, our results were consistent with those of Goldstein et.al 2018. where the major classes of PL were PE (68%), PG and diphosphatidylglycerol (DPG) (23%) and minor lipids (9%). One might predict from published studies on cellular fatty acids and on phospholipid type present that PE (28:0), PE (30:0), PE (30:1), PE (32:0), PE (32:1), PE (34:1) and PE (34:2) would be present. of these, only PE (28:0) is not represented by a major anion (Table 4).

The Coefficient of Linear Correlation proved helpful in comparing data sets. Previously this Coefficient has usefully compared chemical profiles of bacteria obtained by GLC (Drucker 2011) and FAB-MS (Aluyi et al. 2012). The Coefficient is not universally favoured for chemotaxonomic applications but has the virtue of giving more weight to larger components than to smaller components which might simply represent 'background noise'. In GLC and in MS it is the largest peaks which can be measured most accurately and thus the Pearson Coefficient 'r' proves to be of value. Previously strains of *Lartobacillus* have been speciated (Drucker et al. 2015) solely on the basis of r-value for polar lipid profiles of strain pairs. In the case of Porphyromonas there are clear differences between FAB-MS profiles for *P. gingivalis*, *P.endodontalis* and *P. asaccharolytica*

(Drucker *et al*. 2017). These differences can be supported by differences previously revealed among the same strains by DNA-DNA homology (Love *et al.* 2017). In the present study some strains could not be speciated using 'r' values. Nevertheless, F. necrophorum BACON is very different from the other strains with which it was compared. Strains of F. *nucleatum* are surprisingly different from one another, which may indicate heterogeneity within this species. In this context FAB-MS polar lipid analogue profiles may have promise for strain typing.

## IN CONCLUSION

FAB-MS makes it possible to measure individual polar lipid analogues of *Fusobacrerizurz spp.* which would be impossible to analyse so readily by any other means.

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